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   The University of Nottingham
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   Patents ADP number *(if you know it)* 43769270002

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   PEPTIDE PRESENTATIONS FOR HUMAN IMMUNODEFICIENCY DISEASE VACCINES

5. Name of your agent *(if you have one)*

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PEPTIDE PRESENTATIONS FOR HUMAN IMMUNODEFICIENCY DISEASE VACCINES

Field of the Invention

This invention relates to presentations of peptides which mimic the epitopes recognised by antibodies capable of neutralising diverse clinical isolates of the human immunodeficiency virus type 1 (HIV-1). Such peptide presentations may be used as prophylactic or preventative vaccines or for the production of antibodies to be used for the prevention or treatment of HIV-1 infection, or for the production of anti-idiotypic antibodies for the prevention or treatment of HIV-1 infection and AIDS.

With almost 50 million individuals currently infected by the human immunodeficiency virus type 1 (HIV-1) and an estimated 16,000 new infections every day, a potent HIV-1 vaccine is needed to induce both cell-mediated and antibody responses in order to neutralise circulating virus and clear infected cells. Whilst considerable progress has been made towards interventions capable of eliciting cell-mediated immunity, the induction of potent neutralising antibodies remains a major challenge.

Protection mediated by an antibody is correlated to its ability to neutralise primary isolates of HIV-1, as opposed to T-cell-line-adapted laboratory strains. Antibodies neutralising primary isolates are present in only a minority

of patient sera. Even the most potent sera tend to neutralise a limited number of isolates. To date, only a few highly conserved neutralising epitopes have been described, and these epitopes are defined by the human monoclonal antibodies 2F5, 4E10, Z13, 2G12 and b12. Each of these antibodies mediates 90% neutralisation of diverse HIV-1 primary isolates at concentrations that are potentially achievable through vaccination. Significant synergy has been observed when these antibodies are combined. Potent *in vitro* neutralisation is correlated with *in vivo* protection against HIV-1 in various animal models. Therefore a vaccine capable of inducing an antibody response neutralising primary isolates is likely to provide protection against HIV-1 infection and/or disease.

The human monoclonal antibody 2F5 recognises one of the few conserved neutralising epitopes accessible on the gp41 sub-unit of the glycoprotein envelope of primary isolates of HIV-1. The epitope encompasses the sequence of six amino acids

Glu Leu Asp Lys Trp Ala

i.e. E L D K W A in one-letter notation, with the core sequence L D K W identified as the most critical. The 2F5 epitope is well-conserved, with the sequence E L D K W A present in 72 % of primary isolates analysed and the core sequence L D K W expressed by 80 % of isolates. However, not all such isolates are neutralised by the 2F5 antibody and even isolates with the E L D K W A epitope vary significantly in their sensitivity to neutralisation. The 2F5 antibody appears to have some binding requirements outside the E L D K W A motif. Despite the identification of the E L D K W A epitope as a potential immunogen, no vaccine candidate based on this sequence has proven capable of inducing neutralising antibodies against primary isolates of HIV-1.

Discontinuous regions within the gp120 subunit of the glycoprotein envelope of primary isolates of HIV-1 form the epitope recognised by the monoclonal antibody b12. There are indications that the epitope overlaps the CD4-binding domain on the gp120 sub-unit.

Although the epitopes recognised by neutralising antibodies are likely to be present and exposed on candidate vaccines based on gp120/gp160 sub-units, in clinical trials none have been able to elicit broadly neutralising antibody responses. Data from over 2000 subjects participating in phase I and phase II clinical trials of highly purified forms of recombinant, monomeric env vaccines demonstrated these products to be safe and generally capable of inducing humoral responses against HIV-1. However the sera from recipients of such vaccines generally failed to neutralise primary isolates of HIV-1 although the induced antibodies often neutralised T-cell-line-adapted laboratory strains. This is consistent with the minimal impact of recombinant env vaccines on the course of HIV-1 disease as reported in clinical and laboratory analyses of breakthrough infections.

The host immune response to the entire gp120/160 molecule of the glycoprotein envelope is usually focused on the more variable (strain-specific) and more accessible immunodominant epitopes on the monomeric subunits. Although native oligomeric forms of the envelope sub-units might serve as better vaccine candidates, the characteristics of the oligomer may limit its immunogenicity. Several domains of the gp120 sub-unit and a large portion of the gp41 sub-unit are inaccessible for antibody binding, especially in the trimeric form of gp160 present on the virion (virus particle). In addition, the outer exposed surface of gp120 is extensively glycosylated, which shields important epitopes from antibody binding.

It has now been found that peptide mimics of the epitopes recognised by antibodies that neutralise primary isolates of HIV-1 are capable of high affinity and specific binding to the paratopes of these neutralising antibodies and, when presented in an appropriate conformation, are capable of inducing antibodies with similar neutralising profiles to the selecting antibodies.

The inherent difficulty in mimicking conformational rather than linear epitopes poses a significant problem that cannot be overcome using standard epitope-mapping methods such as pepscanning.

Peptides capable of binding to the selecting antibody have been identified among the peptides expressed as fusion proteins in selectively enriched random peptide display libraries. The technique utilises a suitable host (phage or bacterium) which is genetically modified to display 6mer to 40mer peptides of a random nature which can be either linear or constrained in a disulphide loop. Multiple rounds of biopanning then enrich peptides able to bind specifically to the target antibody molecule. This is an iterative process whose success is linked to the extremely high combination of different sequences of amino acids within the inserted peptides presented in the random peptide display library. Peptides identified in this way can mimic conformational as well as linear epitopes and may also mimic interactions with non-proteinaceous (*e.g.* carbohydrate) antigens. New libraries for selection of peptides with improved binding kinetics and affinity may be created by mutagenising the peptide insert present in the selected host.

There is evidence that broadly reactive neutralising antibodies such as 2F5 and b12 have complementarity-determining regions which are composed of more than 6 to 8 amino acid residues, large compared with most other antibodies. This may partly explain why antibodies raised using linear or cyclic peptides exposed on the surface of an immunogen fail to neutralise primary HIV-1 isolates. Partial occlusion of the epitope within a pocket or cleft may select for antibodies with large complementarity-determining regions following immunisation.

According to the present invention, there are provided partially occluded and/or multimeric presentations of peptides which are recognised by HIV-1 neutralising antibodies capable of neutralising diverse clinical isolates of HIV-1. By "partially occluded" is meant a presentation that has a three-dimensional structure that has internally, at or near its base, the epitope that is recognised by the neutralising antibody; *i.e.* a partially occluded presentation is a three-dimensional presentation of one or more neutralising epitopes such that the epitope is located in a pocket or cleft. Such a presentation is better at eliciting antibodies that have the neutralising phenotype.

This invention also refers to the use of such peptide presentations, either alone or in combination with a carrier molecule or as fusion proteins or as part of a chimerical organism, for the induction of antibodies capable of neutralising HIV-1, and to the antibodies thus obtained. This invention also covers the use of such peptide presentations as components of preventative or therapeutic vaccines against HIV-1, or such use of antibodies that arise following immunisation using these peptides.

The invention provides vaccines comprising such peptide presentations. As simple peptides themselves have poor immunogenicity, a peptide epitope may have to be presented in a modified way to facilitate induction of neutralising antibodies. For example, the peptide may be administered with an adjuvant or coupled to a carrier molecule, or it may be expressed as a fusion protein or as a recombinant or chimerical prokaryotic or eukaryotic organism.

The peptide may be used with an appropriate carrier molecule, such as tetanus toxin or keyhole limpet haemocyanin. Alternatively the peptide may be cloned so that it can be expressed in the context of well-characterised fusion proteins, for example thioredoxin or phage proteins. In addition, peptides can be presented in the context of immune-accessible proteins of various organisms to produce a recombinant or chimeric vaccine. The induction of mucosal as well as peripheral antibody responses may result from alterations in the route of administration or of the background organism used to produce the recombinant immunogen. Common recombinant organisms include recombinant modified vaccinia Ankara, *Mycobacterium bovis* BCG and *Salmonella typhimurium*. Immunogenicity of the peptide may also be enhanced by presenting it alongside other immune signals, *e.g.* cytokines, or by modifying the release of the antigen, *e.g.* by controlling its delivery from polymeric microspheres.

The following example illustrates the invention with reference to monoclonal antibody 2F5, which is one of the few known to have the ability to neutralise primary isolates of HIV-1.

Affinity selection of 28mer phage peptides against antibody 2F5

Affinity selection of the pC89/pIF4 type 8+8 phagemid random peptide display library was used in biopanning experiments to identify peptides capable of specific binding to human monoclonal antibody 2F5, which is a human IgG_1 molecule specific for the gp41 sub-units of the glycoprotein envelope of HIV-1. The antibody was derived from a donor infected with asymptomatic HIV-1 (sub-type B) by the fusion of peripheral blood mononuclear cells with CB-57 heteromyeloma cells and the subsequent selection of secreted antibodies against recombinant HIV-1 gp41 and gp160 (A. Buchacher *& al.* 1994. "Generation of human monoclonal antibodies against HIV-1 proteins; electrofusion and Epstein-Barr transformation for peripheral blood lyphocyte immortalisation". *AIDS Research and Human Retroviruses* **10:** 359-369).

The pC89/pIF4 phage vectors carry an ampicillin resistance marker, an ~~fd~~ F1origin of replication and a recombinant gene 8 (g8) expressing random peptides at position six of the mature p8 capsid protein. The displayed peptides are therefore preceded by the amino acids

Ala Glu Gly Glu Phe

(*i.e.* A E G E F in one-letter notation). The expression level of recombinant p8 is under the control of a p*Lac* promoter inducible with isopropylthiogalactoside and can theoretically vary between 1 and 2700 copies per phage. The pIF4 vector is a derivative of pC89 in which the native g8 leader sequence of the M13 phage of *Escherichia coli* is replaced by that of *pel*B. When bacteria containing pC89/pIF4 phagemids are superinfected with M13K07 helper phage, the hybrid M13 phagemid particles are secreted into the culture supernatant.

Affinity selections from the pC89/pIF4 phagemid libraries were performed using published methods (F. Felici *& al.* 1991. "Selection of antibody ligands from a large library of oligopeptides expressed on a multivalent exposition vector". *Journal of Molecular Biology* **222**, 301-310), with modifications as described below. One major modification was the elution of phage binding to

the selecting molecule through competition with an appropriate ligand to favour the isolation of biologically active peptides. Thus for antibodies to the HIV-1 envelope glycoproteins, gp160 was used for elution. An oligomeric gp160 (o-gp160$_{IIIB}$; Autogen Bioclear, Wiltshire, UK) was chosen for these experiments, as this may be more representative of the native form of this protein on the HIV-1 virion. Following this affinity selection, the enriched libraries were expanded in order to reduce the potential for the accidental loss of rare target-binding clones during subsequent rounds.

In brief, affinity selection of pC89 phagemid random peptide display libraries, containing random peptide inserts of 9 and 28 amino acids respectively, was performed in the wells of a maxisorp microtitre plate (Nunc, Roskilde, Denmark). Monoclonal antibody 2F5 was coated to these wells at a concentration of 10.0 to 100 μgml^{-1} by overnight incubation in 100 µl of coating buffer (0.05 M carbonate-bicarbonate, pH 9.6) at 4°C. Wells were then blocked for 1 hour at 4°C with 300 µl of TBS-TB (Tris-buffered saline, pH 7.6 (TBS) containing 0.1% v/v Tween 20 and 1.0 % v/v bovine serum albumin (BSA)). After discarding the blocking solution, wells were washed three times with TBS-T (TBS containing 0.1% v/v Tween 20) before the addition of 1×10^{11} phage from an equimolar mix of pC89 and pIF4 phagemid random peptide display libraries, diluted in 100 µl TBS-T. These phage were allowed to bind immobilised proteins for 1 hour at 25°C before the unbound phage were removed through ten serial washes with TBS-T. Bound phage were eluted with 100 µl of competitor molecule (o-gp160$_{IIIB}$) diluted to 10 μgml^{-1} in TBS.

Following elution, the enriched libraries were expanded by the infection of 1×10^{5} phage into 300 µl of log phase *Escherichia coli* ER2537. The outgrowth of these infected bacteria was then performed by the addition of 1.0 ml Luria broth (LB) containing 0.5 μgml^{-1} ampicillin, followed by incubation at 37°C for 30 minutes. This culture was then plated on LB agar containing 1% glucose and 100 μgml^{-1} of ampicillin in 230 mm^2 bio-assay plates. Glucose was used as a metabolic repressor of the p*Lac* promoter to reduce the

effects of any biological bias associated with the expression of recombinant peptides. After overnight growth at 37°C, bacteria were collected and transferred into 5 ml LB-GA (LB containing 10 % glycerol and 100 μgml^{-1} of ampicillin) using sterile cell-scrapers (Philip Harris Ltd., UK). Bacteria were then filtered through sterile muslin to remove agar and other debris and the filter flushed through with an additional 5 ml LB-GA to collect the remaining cells. The absorbance of this culture was read at 600nm and the concentration of recovered bacteria estimated (1.0 $OD_{600nm} \equiv 3.3\times10^8$ bacteria ml^{-1}).

An appropriate volume of this culture was then transferred to 5 ml of LB containing 100 μgml^{-1} ampicillin to give a final OD_{600nm} of 0.05 and this culture was then grown to an OD_{600nm} of 0.3 at 37°C. Bacteria were then super-infected by the addition of M13K07 helper phage at an MOI of 30 before growing for a further hour at 37°C. Bacteria were transferred to a sterile 250 ml baffled culture flask containing 30 ml LB supplemented with 100 μgml^{-1} ampicillin and 50 μgml^{-1} kanamycin and grown overnight (16 hours) at 37°C. Bacteria were cleared by centrifugation at 10 000×g for 10 minutes and phage subsequently recovered from the supernatant of this culture by the addition of one fifth volume of PEG (20 % w/v polyethylene glycol-8000, 2.5 M sodium chloride) for 1 hour at 4°C and collected by centrifugation at 15000×g for 20 minutes.

Phage pellets were then re-suspended in 1.0 ml TBS before a second precipitation with one fifth volume of PEG, for 1 hour on ice. Phage were subsequently collected by centrifugation at 10 000×g for 20 minutes (MSE Micro Centaur) and re-suspended in a final volume of 200 µl sterile TBS. An input of 1×10^{12} phage was subsequently used as the input to a further two or three rounds of affinity selection. For the final round, reducing the incubation period of the selecting molecule with the enriched libraries from 1 hour to 10 minutes increased the stringency of selection.

Following the final round of affinity selection, individual members of the enriched library populations were isolated and the amino acid sequence of the

displayed peptides was deduced by sequencing of the recombinant DNA encoding them.

The nomenclature used throughout this example to identify clones derived from a phagemid random peptide display library is based on a combination of the size of peptide displayed, the round of enrichment from which clone was derived, and a unique clone number. Thus clone 28.3.1 represents the first clone isolated after three rounds of affinity selection of a 28mer phagemid random peptide display library.

Following affinity selection of the pC89/pIF4 gene 8 28mer phagemid random peptide display libraries, the majority of selected clones had a primary amino acid sequence that shared homology to the 2F5-epitope. The random peptide insert for five out of twelve clones was of the single sequence of 28 amino acids represented by phage 28.3.1 and shown in the following formula

Glu	Trp	**Glu**	Asp	Val	Glu	Phe	**Glu**	**Leu**	**Asp**	***Arg***	**Trp**	**Ala**
Leu	Arg	Ser										
1	2	3	4	5	6	7	8	9	10	11	12	13
14	15	16										
Cys	Cys	Pro	Val	Glu	Gly	Ala	**Trp**	Arg	**Trp**	Arg	Gly	
17	18	19	20	21	22	23	24	25	26	27	28	

i.e. E W **E** D V E F **E L D *R*** **W** A L R S C C P V E G A **W** R **W** R G in one-letter notation.

(Compared with the gp41 sequence of the HxB2 strain, identical amino acids are shown in **bold** type and related amino acid in ***bold italic*** type. The adjacent cysteine residues are underlined.)

The sequence of 28 amino acids in this phage peptide has two striking features: (i) there are regions of homology to the sequence of the sub-unit gp41 of the HIV-1 envelope glycoprotein outside the previously described epitope; and (ii) there are adjacent cysteine residues within the peptide sequence. This phage peptide may have conformational complexity not seen in the smaller peptide inserts.

Disulphide bridging in phage peptide 28.3.1

The adjacent cysteine residues in the 28mer phage peptide 28.3.1 may form inter-molecular covalently-linked complexes leading ultimately to a partially occluded presentation of the epitope. This was investigated by carrying out sodium dodecyl sulphate/polyacrylamide gel electrophoresis / western blotting of the phage peptide, with a phage peptide selected against monoclonal antibody 2G12-as negative control and in some cases a 9-mer (instead of 28mer) phage peptide selected against monoclonal antibody 2F5 as positive control. Proteins were resolved by sodium dodecyl sulphate/polyacrylamide gel electrophoresis.

Acrylamide resolving gels (8.0 to 16.0 %) were cast in an Atto blot gel apparatus (Atto, Japan) assembled according to the manufacturers instructions. Resolving gel solution (10 ml) was prepared as described by E. Harlow & D. Lane in "Antibodies: a laboratory manual" (Cold Spring Harbour Laboratory, Cold Spring Harbour, New York. 1988) by combining the appropriate volumes of easigel (30.0 % acrylamide:bisacrylamide solution (ratio 37.5:1), Anachem), 1.5 M Tris (pH 8.8), 10.0% sodium dodecyl sulphate, 10.0% ammonium persulphate, N,N,N',N'-tetramethylethylenediamine and distilled water. Gels were poured immediately, overlaid with distilled water and allowed to polymerise. Once polymerised, the surface of the resolving gel was flushed with distilled water before overlaying with a 5.0 % polyacrylamide stacking gel prepared by combining the appropriate volumes of easigel, 1.0 M Tris (pH 6.8), 10.0% sodium dodecyl sulphate, 10.0% ammonium persulphate, N,N,N',N'-tetramethylethylenediamine and distilled water.

Aliquots containing 5×10^{10} phage particles were either untreated or treated with 3 µl of 0.27 M N-ethylmaleimide (Sigma) or 3 µl of 0.54 M dithiothreitol (Sigma), incubated for one hour at 4°C, then mixed with an equal volume of 2× Laemmli buffer (4% sodium dodecyl sulphate, 200 mM dithiothreitol, 120 mM Tris (pH 6.8), 0.002% bromophenol blue) and heated to 85°C for 5 minutes (M.B. Zwick *& al.* 2001. "Identification and characterisation of a

peptide that specifically binds the human immunodeficiency virus type 1 antibody b12". *Journal of Virology* **75**, 6692-6699). Aliquots of 10.0 µl were loaded into the wells of the polymerised stacking gel then resolved by electrophoresis at a constant voltage of 150 V for 1.0 to 2.0 hours in Tris-glycine buffer (25.0 mM Tris, 250 mM glycine, 0.1% sodium dodecyl sulphate). To assess the molecular weight of the proteins detected by western blot, all gels were run with either high or low range rainbow molecular weight markers (Amersham-Pharmacia) as appropriate.

For immunological detection, resolved proteins were transferred to polyvinylidene difluoride membranes that had been equilibrated by sequential incubations with 100% ethanol and transfer buffer (48 mM Tris, 39 mM glycine, 0.037% v/v sodium dodecyl sulphate, 20 % v/v methanol). Transfer was performed using the Trans-Blot SD semi-dry transfer cell apparatus (Biorad) run at 25 V for 1.5 hours. Membranes were then blocked with Tris-buffered saline, pH 7.6 (TBS) containing 0.1% Tween 20 and 3% dry milk powder (TBS-TM) for 1 hour at 25°C before overnight incubation with primary antibodies diluted to 1.0 $\mu g ml^{-1}$ in TBS-T. Primary antibodies used were 2F5, a rabbit anti-phage polyclonal serum (Sigma) and mouse serum raised against the 2F5 epitope displayed in the context of *Escherichia coli* MalE protein (E. Coeffier *& al.* 2000. "Antigenicity and immunogenicity of the HIV-1 gp41 epitope ELDKWA inserted into permissive sites of the MalE protein". *Vaccine* **19**, 684-693). After washing with Tris-buffered saline, pH 7.6 (TBS) containing 0.1% Tween 20 (TBS-T), membranes were incubated with the appropriate horse radish peroxidase (HRP)-conjugated antibody diluted to 1:2000 in TBS-T for 2 hours at 25°C. Following a final series of washes, the membrane was either incubated with 3,3'-diaminobenzidine before drying briefly under a vacuum at 85°C or incubated with electrochemiluminescent substrate (Amersham) for 1 minute before exposing to X-ray film for 1 to 30 minutes.

The rabbit-anti-phage serum (of rabbit anti-fd antibody) demonstrated the presence of phage proteins in the untreated 28.3.1 phage peptide and negative

control phage peptide preparations. Probing the blotted native untreated protein samples with 2F5 antibody showed the presence of 2F5-reactive p8 fusion protein only in the 28.3.1 clone. There were two molecular weight forms of the 28.3.1 protein apparent, one with a predicted molecular mass of approximately 5 kDa and the other with a predicted mass of approximately 15 kDa. The latter is assumed to be a trimeric form of the p8 fusion protein. A similar pattern was observed for the protein treated with N-ethyl maleimide. This suggests that the trimeric form of the protein exists on the surface of the phage rather than forming after lysis of the phage particles. Following treatment with dithiothreitol only the predicted monomeric form of the 28.3.1 p8 fusion protein was detected, suggesting that the trimer was indeed formed by disulphide bridging between three adjacent monomeric p8 units. No multimeric complexes of the p8 fusion peptide were seen in any other phage clones generated during the biopanning experiments nor did these clones possess adjacent cysteine residues in the peptides inserts.

Immunoassay of phage peptide 28.3.1

To assess the accessibility of the predicted 2F5 epitope on the monomeric and trimeric p8 fusion protein present in clone 28.3.1, further western blots were performed using mouse sera raised against the 2F5 epitope expressed in MalE protein; these sera are known not to be able to neutralise primary isolates of HIV-1 (Coeffier *& al.*, 2000). The mouse sera detected the monomeric presentation of the untreated native 28.3.1 p8 fusion protein and 28.3.1 p8 fusion protein treated with N-ethyl maleimide but not the trimeric form. As expected, probing western-blotted 28.3.1 phage protein treated with dithiothreitol revealed the presence of a single band representative of monomeric p8 fusion protein.

In enzyme immunoassays the 28.3.1 clone demonstrated high and specific reactivity to the 2F5 antibody compared to an HIV-1-specific 2G12 antibody as negative control.

To perform the immunoassays, antibodies were diluted to between 1.0 and 100 μgml^{-1} in 50 µl carbonate bicarbonate buffer and coated directly into the wells of a microtitre plate by overnight incubation at 4°C. Following blocking with TBS-TM, approximately 1×10^{11} phage particles were added to the wells and allowed to bind for 1 to 2 hours. Bound phage were then detected by incubation with either the rabbit anti-fd antibody or a serum raised against wild-type M13 phage by immunisation of mice, both diluted to 1:1000. The binding of these antibodies was then detected by sequential incubations with an appropriate secondary antibody and substrate and the absorbance at 490 nm recorded as described. The cut-off above which phage were considered to be reactive with an antibody or protein was defined as the mean OD_{490nm} of reactivity to wild-type M13 plus three standard deviations. The absorbance of all 17 samples of the 2G12 antibody was below the cut-off (OD_{490nm} 0.0125) whereas only three of the 2F5 antibody samples were below the cut-off , one showed OD_{490nm} 0.25, and the OD_{490nm} of the remaining thirteen ranged from 0.50 to 1.60.

Conclusion

Taken together, the data in this example show that the 28.3.1 p8 phage fusion protein, containing the peptide sequence displayed above, exists on the surface of the phage as both a monomer and a multimer promoted by inter-p8 disulphide bonds between the cysteine residues present at positions 17 and 18. This multimeric presentation may partially occlude the 2F5 epitope from non-neutralising mouse antibodies, although the mouse serum does recognise the monomeric presentation of the epitope. By contrast the epitope is accessible to neutralising 2F5 antibody on both the monomer and the trimer.

It appears therefore that partially occluded and/or multimeric presentations of HIV-1-neutralising epitopes preferentially select for antibodies with a virus-neutralising phenotype, and that they are therefore better immunogens in terms of their potential for HIV vaccines or treatments than the linear or surface-exposed presentations of these epitopes previously described. It further

appears that such a presentation may allow simultaneous contact between the antibody and the epitope present on different sub-units, or that such a presentation mirrors the presentation of the epitope on the trimeric gp41 sub-units of the glycoprotein envelope of primary isolates of HIV-1.

The three-dimensional structure (probably a barrel / cylindrical / helical shape) is formed by the three linked peptide monomers (in this case as presented on the phage major coat protein p8, but there could be a similar presentation on any carrier molecule). The depth at which the epitope is located in a pocket or cleft within the cylinder / barrel / helix may be variable, but the depth and the primary amino acid sequence are each sufficient to encourage the selection of monoclonal antibodies with complementarity-determining regions that are sufficiently long to interact with the epitope as it is present on native primary HIV-1 isolates. In the case of the epitope described in the example above, the 28 amino acid sequence is preceded at the N-terminal end of the phage gene 8 protein by the amino acid sequence Ala Glu Gly Glu Phe, making the total length of the amino acid peptide upstream of the adjacent cysteine residues 21 amino acids long.

Several possible disulphide-bridged states exist in the multimeric presentation. These can be assigned according to the sub-unit the cysteine residue is located on and the relative position of the cysteine residue on the primary amino acid sequence. By way of illustration, the trimer presented in the 28.3.1 p8 phage fusion protein might for example have disulphide bridging between the cysteine at position 16 on sub-unit I (I-C_{16}) and the cysteine at position 16 on sub-unit III (III-C_{16}) together with bridging between the cysteine at position 17 on sub-unit III (III-C_{17}) and the cysteine at position 16 on sub-unit II (II-C_{16}) and together with bridging between the cysteine at position 17 on sub-unit II (II-C_{17}) and the cysteine at position 17 on sub-unit I (I-C_{17}). (The trimer might thus be represented as I- C_{16} -S-S- III-C_{16}, III-C_{17} –S-S- II-C_{16}, II-C_{17} –S-S- I-C_{17}.) This invention relates to all possible combinations of partially and completely disulphide-bonded multimeric forms.

The partially occluded presentation should be such as to exclude access of the whole immunoglobulin molecule or B-cell to the epitope, thereby preferentially selecting antibodies that have complementarity-determining regions that would be capable of accessing or interacting with the epitope as presented on the native virion (virus particle).

A partially occluded presentation according to the invention may also be used to present the native epitope as it appears on virus isolates or other linear peptide sequences shown to be reactive with a neutralising monoclonal antibody. The following example shows a disulphide-bridged presentation of the HIV-1 (strain HxB2) sequence encompassing the 2F5 epitope:

Ala Glu Gly Glu Phe Ala Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp

Lys Trp Ala Ser Leu Trp Cys Cys Phe Asn Ile Thr Asn Trp Leu Trp

carrier

i.e. **A E G E F A** K N E Q E L L E L D K W A S L W C C F N I T N W L W– *carrier* in one-letter notation. The amino acid residues forming an additional N-terminal flanking sequence to help maintain the depth of the pocket / cleft within the trimer are in bold type.

Whilst the primary amino acid sequences of the epitopes or mimotopes recognised by monoclonal antibodies 2F5 and b12 have previously been described, there is novelty in the method of presentation of these epitopes. This invention also covers such a presentation, as a trimeric or multimeric and/or partially occluded epitope, of epitopes recognised by other HIV-1 neutralising antibodies such as 4E10, Z13 and 2G12 capable of neutralising diverse clinical isolates of HIV-1.

The epitopes defined and recognised by the Z13 and 4E10 monoclonal antibodies may similarly be presented in a multimeric and / or partially occluded presentation, either alone or in combination with each other and the 2F5 epitope. For example, the following is a presentation of the HIV-1 gp41

fragment encompassing the 2F5 epitope (shown in bold italic type) and 4E10/Z13 (shown in bold type underlined) epitopes).

Asn Gln Gln Glu Lys Asn Glu Gln Glu Leu Leu Glu ***Leu Asp Lys Trp***

Ala Ser Leu Trp **<u>Asn</u>** **<u>Trp</u>** **<u>Phe</u>** **<u>Asn</u>** **<u>Ile</u>** **<u>Thr</u>** Asn Trp Cys Cys *carrier*

i.e. N Q Q E K N E Q E L L E ***L D K W A S*** L W **<u>N W F N I T</u>** N W C C–carrier in one-letter notation.

Also, the recently described epitope recognised by the monoclonal antibody b12 which neutralises primary HIV-1 isolates may be similarly modified by insertion of an additional cysteine residue immediately downstream of the reported cysteine residue in order to promote a multimeric presentation by inter-chain disulphide bridging. Addition of residues at the N-terminus leads to further occlusion of the epitope and thus to preferential selection of antibodies with large complementarity-determining regions, and hence of a neutralising phenotype. Both of these modifications to the b12 epitope are shown in the following formula

Ala	**Glu**	**Gly**	**Glu**	**Phe**	**Ala**	**Ala**	**Ala**	His	Glu	Arg	Ser	Tyr
Met	Phe	Ser									1	2
3	4	5	6	7	8							

Asp	Leu	Glu	Asn	Arg	<u>Cys</u>	***<u>Cys</u>***	Ile – *carrier*
9	10	11	12	13	14		15

i.e. **A E G E F A A A A** H E R S Y M F S D L E N R **<u>C</u>** ***<u>C</u>*** I*carrier* in one-letter notation. The b12 epitope sequence (to which the numbering relates) is shown in full, with the additional cysteine residue in ***bold italic*** type and the additional N-terminal residues in **bold** type. Cysteine residues are underlined.

In all of the trimeric or multimeric presentations of the-epitopes, the conformation may be stabilised by inter-chain disulphide bridging of the reactive peptides or by other chemical means to generate a three-dimensional structure similar to that created by the disulphide-bridged peptides.

The multimeric and/or partially occluded presentations of the reactive peptides according to the invention may be used to induce neutralising antibodies in an immunised host organism (including a suitable cell line). Such presentations may also be used to discriminate between antibodies that neutralise primary HIV-1 isolates, and those that do not. The presentations of the invention and antibodies generated by immunisation with such presentations may be used for the prevention or treatment of HIV-1 infection and AIDS, or for the production of anti-idiotypic antibodies for the prevention or treatment of HIV-1 infection and AIDS irrespective of whether or not that immunising antibody is neutralising.

CLAIMS

1. A partially occluded and/or multimeric presentation of a peptide which is recognised by an HIV-1 neutralising antibody capable of neutralising diverse clinical isolates of HIV-1.

2. A trimeric presentation of a peptide as claimed in claim 1.

3. A multimeric presentation of a peptide as claimed in claim 1 or claim 2 which is stabilised by inter-chain disulphide bridging of the reactive peptides or by other chemical means to generate a three dimensional structure similar to that created by the disulphide-bridged peptides.

4. The use of a partially occluded and/or multimeric presentation of a peptide as claimed in any one of claims 1 to 3 to induce neutralising antibodies in an immunised host organism.

5. An antibody obtained in accordance with claim 4 capable of neutralising diverse clinical isolates of HIV-1.

6. A vaccine for the prevention or treatment of HIV-1 infection and AIDS which comprises a partially occluded and/or multimeric presentation of a peptide as claimed in any one of claims 1 to 3.

7. The use of a partially occluded and/or multimeric presentation of a peptide as claimed in any one of claims 1 to 3 as a means of generating an anti-idiotypic antibody for the prevention or treatment of HIV-1 infection and AIDS irrespective of whether or not that immunising antibody is neutralising.

ABSTRACT

PEPTIDE PRESENTATIONS FOR HUMAN IMMUNODEFICIENCY DISEASE VACCINES

Partially occluded and/or multimeric presentations of peptides mimic the epitopes recognised by antibodies capable of neutralising diverse clinical isolates of the human immunodeficiency virus type 1 (HIV-1). By "partially occluded" is meant a presentation that has a three-dimensional structure (probably a barrel / cylindrical / helical shape) generated by inter-chain disulphide bridging or other means that has internally, at or near its base, the epitope that is recognised by the neutralising antibody; *i.e.* a partially occluded presentation is a three-dimensional presentation of one or more neutralising epitopes such that the epitope is located in a pocket or cleft. Such presentations are better at eliciting antibodies that have the neutralising phenotype, and may be used as vaccines or to produce antibodies for the prevention or treatment of HIV-1 infection.